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(57) Abstract: The present invention provides a biomarker that is predictive for the response to treatment with an EGFR inhibitor in cancer patients.



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PREDICTIVE MARKER FOR EGFR INHIBITOR TREATMENT

The present invention provides a biomarker that is predictive for the response to treatment with an EGFR inhibitor in cancer patients

A number of human malignancies are associated with aberrant or over-expression of the epidermal growth factor receptor (EGFR). EGF, transforming growth factor α (TGF- α), and a number of other ligands bind to the EGFR, stimulating autophosphorylation of the intracellular tyrosine kinase domain of the receptor. A variety of intracellular pathways are subsequently activated, and these downstream events result in tumour cell proliferation in vitro. It has been postulated that stimulation of tumour cells via the EGFR may be important for both tumour growth and tumour survival in vivo.

Early clinical data with Tarceva, an inhibitor of the EGFR tyrosine kinase, indicate that the compound is safe and generally well tolerated at doses that provide the targeted effective concentration (as determined by preclinical data). Clinical phase I and II trials in patients with advanced disease have demonstrated that Tarceva has promising clinical activity in a range of epithelial tumours. Indeed, Tarceva has been shown to be capable of inducing durable partial remissions in previously treated patients with head and neck cancer, and NSCLC (Non small cell lung cancer) of a similar order to established second line chemotherapy, but with the added benefit of a better safety profile than chemo therapy and improved convenience (tablet instead of intravenous [i.v.] administration). A recently completed, randomised, double-blind, placebo-controlled trial (BR.21) has shown that single agent Tarceva significantly prolongs and improves the survival of NSCLC patients for whom standard therapy for advanced disease has failed.

Tarceva (erlotinib) is a small chemical molecule; it is an orally active, potent, selective inhibitor of the EGFR tyrosine kinase (EGFR-TKI).

The human epidermal growth factor receptor (EGFR) is a tyrosine-kinase (TK) receptor that plays an important role in several cellular signaling pathways, including those involved in proliferation and survival. EGFR has a well established role in several solid tumor types and constitutes a clinically validated target for anticancer therapies. Erlotinib (OSI-774, Tarceva[®]) is a potent, orally available EGFR tyrosine-kinase inhibitor (TKI) that blocks EGFR-mediated intracellular signaling and induces tumor cell cycle arrest.

Erlotinib is approved by the US Food and Drug Administration (FDA) and the European Medicines Agency for treatment of patients with locally advanced or metastatic non-small-cell lung cancer (NSCLC) after failure of at least one prior chemotherapy regimen. It is also approved by the US FDA for treatment, in combination with gemcitabine, of locally advanced
5 unresectable or metastatic pancreatic cancer. Several studies in NSCLC have shown that erlotinib and gefitinib (Iressa[®]; another EGFR TKI) produce radiographic responses in approximately 10% of patients treated in the second- or third-line setting. Clinical characteristics associated with tumor response have been extensively described and include: female gender, never-smoking status, adenocarcinoma histology, Asian ethnic origin, *EGFR* gene amplification
10 and the presence of specific mutations in the EGFR TK domain. The study of molecular biomarkers of erlotinib response showed that the incidence of *EGFR* mutations in lung cancer was 22%.

EGFR has been implicated in the tumorigenesis of head and neck squamous-cell carcinoma (HNSCC). The antitumor activity of erlotinib, alone or in combination with cisplatin, has been
15 demonstrated *in vivo* using murine xenografts of a human HNSCC cell line. Furthermore, in a phase I study, erlotinib produced stable disease lasting for 15 months in one patient with HNSCC. In a subsequent phase II study, erlotinib was well tolerated in a heavily-pretreated population of patients with HNSCC and produced disease stabilization in 38% of cases, with a median duration of 16.1 weeks. Recently, cetuximab (Erbix[®]), an antibody targeting EGFR, showed
20 encouraging results in combination with radiotherapy for treatment of locally advanced head and neck squamous cell carcinomas that led to its approval in this indication. A phase III study published by Burtneiss et al. demonstrated that the combination of cisplatin and cetuximab was active in the first line treatment of recurrent HNSCC. EGFR-targeted molecules are likely to become a therapeutic option in HNSCC; however there is a clear medical need to identify which
25 patients are most likely to benefit from therapy with EGFR inhibitors. Contrary to NSCLC, few factors predictive of response have been identified in HNSCC. Numerous teams have assessed the existence of EGFR TK mutations in this disease but they seem to be rare at least in Caucasian patients. Development and intensity of skin rash caused by anti-EGFR therapies have been correlated with improved survival. Recently, Agulnik et al investigated tumor and skin
30 tissue samples to identify biomarkers correlated with response to treatment with erlotinib and cisplatin. Their results suggest that HNSCC patients with high gene copy number of EGFR gene may have higher response rate. Among the EGFR signaling proteins investigated before and after treatment, the decrease of phosphorylated EGFR (p-EGFR) in both normal and tumor tissue

was linked with increased overall survival indicating that decrease in p-EGFR may represent a potential surrogate marker for outcome. However, the results were obtained for erlotinib in combination with cisplatin in patients participating to phases I and II who were already heavily pretreated. Such biomarkers should be examined in patients treated with erlotinib as a single agent in order to characterize the clinical response to this particular therapy.

It has long been acknowledged that there is a need to develop methods of individualising cancer treatment. With the development of targeted cancer treatments, there is a particular interest in methodologies which could provide a molecular profile of the tumour target, (i.e. those that are predictive for clinical benefit). Proof of principle for gene expression profiling in cancer has already been established with the molecular classification of tumour types which are not apparent on the basis of current morphological and immunohistochemical tests.

Therefore, it is an aim of the present invention to provide expression biomarkers that are predictive for response to EGFR inhibitor treatment in cancer patients.

In a first object, the present invention provides an *in vitro* method of predicting the response of a cancer patient to treatment with an EGFR inhibitor comprising the steps: determining the expression level of at least one gene selected from the group consisting of GSTA4, CRYAB, PRDX2, NUPR1, ELF3, EPHX1, TBL1X, ABCC5, CEBPD, SMARCA4, ABCC1, INHBB, TP53BP2, EI24 in a tumour sample of a patient and comparing the expression level of the at least one gene to a value representative of an expression level of the at least one gene in tumours of a non responding patient population, wherein a lower expression level of the at least one gene in the tumour sample of the patient is indicative for a patient who will respond to the treatment.

In a second object, the present invention provides an *in vitro* method of predicting the response of a cancer patient to treatment with an EGFR inhibitor comprising: determining the expression level of at least one gene selected from the group consisting of THBS1, SERPINE2, AMIGO2, LEPREL1, VEGFC, HSPA2 in a tumour sample of a patient and comparing the expression level of the at least one gene to a value representative of an expression level of the at least one gene in a non responding patient population, wherein a higher expression level of the at least one gene in the tumour sample of the patient is indicative for a patient who will respond to the treatment.

The term "a value representative of an expression level of the at least one gene in tumours of a non responding patient population" refers to an estimate of the mean expression level of the marker gene in tumours of a population of non responding patients.

In a preferred embodiment, the expression level of the at least one gene is determined by microarray technology or other technologies that assess RNA expression levels like quantitative RT-PCR, or by any method looking at the expression level of the respective protein, e.g. immunohistochemistry (IHC). The construction and use of gene chips are well known in the art. see, U. S. Pat Nos. 5,202,231; 5,445,934; 5,525,464; 5,695,940; 5,744,305; 5,795, 716 and 1 5,800,992. See also, Johnston, M. Curr. Biol. 8:R171-174 (1998); Iyer VR et al., Science 283:83-87 (1999). Of course, the gene expression level can be determined by other methods that are known to a person skilled in the art such as e.g. northern blots, RT-PCR, real time quantitative PCR, primer extension, RNase protection, RNA expression profiling.

10 In a further preferred embodiment, the expression level of at least two genes is determined, preferably of at least three genes.

The genes of the present invention can be combined to biomarker sets. Biomarker sets can be built from any combination of biomarkers listed in Table 2 to make predictions about the effect of EGFR inhibitor treatment in cancer patients. The various biomarkers and biomarkers 15 sets described herein can be used, for example, to predict how patients with cancer will respond to therapeutic intervention with an EGFR inhibitor.

In a preferred embodiment, the marker is gene GSTA4 and shows typically between 1.3 and 3.5 or more fold lower expression in the tumour sample of the responding patient compared to a value representative of the expression level of the gene GSTA4 in tumours of a non 20 responding patient population.

In a preferred embodiment, the marker is gene CRYAB and shows typically between 1.1 and 3.5 or more fold lower expression in the tumour sample of the responding patient compared to a value representative of the expression level of the gene CRYAB in tumours of a non responding patient population.

25 In a preferred embodiment, the marker is gene PRDX2 and shows typically between 1.4 and 2.8 or more fold lower expression in the tumour sample of the responding patient compared to a value representative of the expression level of the gene PRDX2 in tumours of a non responding patient population.

In a preferred embodiment, the marker is gene NUPR1 and shows typically between 1.3 30 and 3.0 or more fold lower expression in the tumour sample of the responding patient compared to a value representative of the expression level of the gene NUPR1 in tumours of a non responding patient population.

In a preferred embodiment, the marker is gene ELF3 and shows typically between 1.1 and 3.3 or more fold lower expression in the tumour sample of the responding patient compared to a value representative of the expression level of the gene ELF3 in tumours of a non responding patient population.

5 In a preferred embodiment, the marker is gene EPHX1 and shows typically between 1.1 and 3.0 or more fold lower expression in the tumour sample of the responding patient compared to a value representative of the expression level of the gene EPHX1 in tumours of a non responding patient population.

10 In a preferred embodiment, the marker is gene TBL1X and shows typically between 1.2 and 2.8 or more fold lower expression in the tumour sample of the responding patient compared to a value representative of the expression level of the gene TBL1X in tumours of a non responding patient population.

15 In a preferred embodiment, the marker is gene ABCC5 and shows typically between 1.1 and 3.0 or more fold lower expression in the tumour sample of the responding patient compared to a value representative of the expression level of the gene ABCC5 in tumours of a non responding patient population.

20 In a preferred embodiment, the marker is gene CEBPD and shows typically between 1.2 and 2.5 or more fold lower expression in the tumour sample of the responding patient compared to a value representative of the expression level of the gene CEBPD in tumours of a non responding patient population.

In a preferred embodiment, the marker is gene SMARCA4 and shows typically between 1.3 and 2.2 or more fold lower expression in the tumour sample of the responding patient compared to a value representative of the expression level of the gene SMARCA4 in tumours of a non responding patient population.

25 In a preferred embodiment, the marker is gene ABCC1 and shows typically between 1.1 and 2.7 or more fold lower expression in the tumour sample of the responding patient compared to a value representative of the expression level of the gene ABCC1 in tumours of a non responding patient population.

30 In a preferred embodiment, the marker is gene INHBB and shows typically between 1.1 and 2.4 or more fold lower expression in the tumour sample of the responding patient compared to a value representative of the expression level of the gene INHBB in tumours of a non responding patient population.

In a preferred embodiment, the marker is gene TP53BP2 and shows typically between 1.2 and 1.8 or more fold lower expression in the tumour sample of the responding patient compared to a value representative of the expression level of the gene TP53BP2 in tumours of a non responding patient population.

5 In a preferred embodiment, the marker is gene EI24 and shows typically between 1.1 and 1.8 or more fold lower expression in the tumour sample of the responding patient compared to a value representative of the expression level of the gene EI24 in tumours of a non responding patient population.

10 In a preferred embodiment, the marker is gene THBS1 and shows typically between 1. and 3.6 or more fold higher expression in the tumour sample of the responding patient compared to a value representative of the expression level of the gene THBS1 in tumours of a non responding patient population.

15 In a preferred embodiment, the marker is gene SERPINE2 and shows typically between 1.1 and 4.6 or more fold higher expression in the tumour sample of the responding patient compared to a value representative of the expression level of the gene SERPINE2 in tumours of a non responding patient population.

20 In a preferred embodiment, the marker is gene AMIGO2 and shows typically between 1.1 and 4.9 or more fold higher expression in the tumour sample of the responding patient compared to a value representative of the expression level of the gene AMIGO2 in tumours of a non responding patient population.

In a preferred embodiment, the marker is gene LEPREL1 and shows typically between 1.1 and 4.9 or more fold higher expression in the tumour sample of the responding patient compared to a value representative of the expression level of the gene LEPREL1 in tumours of a non responding patient population.

25 In a preferred embodiment, the marker is gene VEGFC and shows typically between 1.2 and 4.6 or more fold higher expression in the tumour sample of the responding patient compared to a value representative of the expression level of the gene VEGFC in tumours of a non responding patient population.

30 In a preferred embodiment, the marker is gene HSPA2 and shows typically between 1.3 and 4.3 or more fold higher expression in the tumour sample of the responding patient compared to a value representative of the expression level of the gene HSPA2 in tumours of a non responding patient population.

The genes of the present invention can be combined to biomarker sets. Biomarker sets can be built from any combination of biomarkers listed in Table 2 to make predictions about the effect of EGFR inhibitor treatment in cancer patients. The various biomarkers and biomarkers sets described herein can be used, for example, to predict how patients with cancer will respond to therapeutic intervention with an EGFR inhibitor.

The term "gene" as used herein comprises variants of the gene. The term "variant" relates to nucleic acid sequences which are substantially similar to the nucleic acid sequences given by the GenBank accession number. The term "substantially similar" is well understood by a person skilled in the art. In particular, a gene variant may be an allele which shows nucleotide exchanges compared to the nucleic acid sequence of the most prevalent allele in the human population. Preferably, such a substantially similar nucleic acid sequence has a sequence similarity to the most prevalent allele of at least 80%, preferably at least 85%, more preferably at least 90%, most preferably at least 95%. The term "variants" is also meant to relate to splice variants.

The EGFR inhibitor can be selected from the group consisting of gefitinib, erlotinib, PKI-166, EKB-569, GW2016, CI-1033 and an anti-erbB antibody such as trastuzumab and cetuximab.

In another embodiment, the EGFR inhibitor is erlotinib.

In yet another embodiment, the cancer is head and neck squamous-cell carcinoma (HNSCC).

Techniques for the detection and quantitation of gene expression of the genes described by this invention include, but are not limited to northern blots, RT-PCR, real time quantitative PCR, primer extension, RNase protection, RNA expression profiling and related techniques. These techniques are well known to those of skill in the art see e.g. Sambrook J et al., Molecular Cloning: A Laboratory Manual, Third Edition (Cold Spring Harbor Press, Cold Spring Harbor, 2000).

Techniques for the detection of protein expression of the respective genes described by this invention include, but are not limited to immunohistochemistry (IHC).

In accordance with the invention, cells from a patient tissue sample, e.g. a tumour or cancer biopsy can be assayed to determine the expression pattern of one or more biomarkers. Success or failure of a cancer treatment can be determined based on the biomarker expression pattern of the cells from the test tissue (test cells), e.g., tumour or cancer biopsy, as being relatively similar or different from the expression pattern of a control set of the one or more biomarkers. In the context of this invention, it was found that the genes listed in table 2 are up-

regulated i.e. show a higher expression level, in tumours of patients who respond to the EGFR inhibitor treatment compared to tumours of patients who do not respond to the EGFR inhibitor treatment. Thus, if the test cells show a biomarker expression profile which corresponds to that of a patient who responded to cancer treatment, it is highly likely or predicted that the individual's cancer or tumour will respond favourably to treatment with the EGFR inhibitor. By contrast, if the test cells show a biomarker expression pattern corresponding to that of a patient who did not respond to cancer treatment, it is highly likely or predicted that the individual's cancer or tumour will not respond to treatment with the EGFR inhibitor.

The biomarkers of the present invention i.e. the genes listed in table 2 are a first step towards an individualized therapy for patients with cancer, in particular patients with head and neck cancer. This individualized therapy will allow treating physicians to select the most appropriate agent out of the existing drugs for cancer therapy, in particular head and neck cancer. The benefit of individualized therapy for each future patient are: response rates / number of benefiting patients will increase and the risk of adverse side effects due to ineffective treatment will be reduced.

In a further object the present invention provides a therapeutic method of treating a cancer patient identified by the in vitro method of the present invention. Said therapeutic method comprises administering an EGFR inhibitor to the patient who has been selected for treatment based on the predictive expression pattern of at least one of the genes listed in table 2. A preferred EGFR inhibitor is erlotinib and a preferred cancer to be treated is head and neck cancer.

Short description of the figures

Figure 1 shows the study design.

Experimental part

Rationale for the Study and Study Design

Patients

Patients were eligible if they had non-metastatic, histologically-confirmed HNSCC (stage $\geq$ T2N x M0), and were candidates for first-line curative surgical treatment or had been scheduled for surgery by necessity (neck nodes dissection for bulky lymphadenopathies prior to radiotherapy). Other eligibility criteria included: WHO performance status $\leq$ 2; able to swallow food; aged $>$ 18 years; and provision of written informed consent.

Patients were not eligible if they had relapsed after radiotherapy, or if they had recent massive gastrointestinal hemorrhage, the presence of a medical contra-indication in the form of a major impairment of general condition, or an ongoing unmanaged serious infectious disease or major metabolic disorder. Other exclusion criteria included: neutrophil count $< 1 \times 10^9/\text{L}$ or platelet count $< 75 \times 10^9/\text{L}$ at study entry; bilirubin > 1.5 -fold above the upper limit of normal; and kidney failure (glomerular filtration rate, calculated using Cockcroft's formula, of $< 40\text{mL}/\text{min}$). Pregnant women and women of childbearing potential were also excluded.

Treatment Plan

After diagnosis, patients underwent routine pan-endoscopy. Treatment with oral erlotinib 150 mg/day (F. Hoffmann-La Roche, Basel, Switzerland) was started the following day. Patients were treated for a variable period ranged from 18 to 30 days, corresponding to the time between pan-endoscopy and surgical resection (see figure 1). In the event of grade 2 diarrhea or skin rash that was symptomatically unacceptable to the patient, treatment was withheld until resolution to grade 1, and then erlotinib was restarted at a dose of 100 mg/day. If toxicity reoccurred, erlotinib was stopped.

Clinical Evaluation

Collection of a full medical history, physical examination, electrocardiogram, and laboratory tests were performed at baseline. Computed tomography imaging of the involved site and systematic radiologic chest evaluation were performed within 1 month of enrolment to ensure that no lung metastases were present. Toxicities were evaluated at each visit and graded using the National Cancer Institute Common Toxicity Criteria, version 2.0. Tumor response was assessed using CT scans taken before and after treatment (on the day before surgery). CT scans to confirm response were not possible as the patients were operated upon. Due to the very short treatment period, patients with tumor shrinkage of more than 25% were arbitrarily considered as responders.

Tissue Biopsies

Tumor tissue biopsies were collected both before and after treatment. Samples were snap frozen in liquid nitrogen and kept at -80°C .

Aim of the expression profiling study with Affymetrix chips.

To study genomic expression of tumors before and after treatment with Affymetrix Human Genome U133A GeneChip®.

These experiments have been realized in 39 patients. Table 1 shows the clinical characteristics of the 39 patients included for genomic profiling study.

	Mean (standart deviation)	Range
Age (years)	55,9 (9,9)	39 - 83
Gender (M/F)	36/3	
Response to treatment (R/NR)	9/30	
Site of primary tumor		
Oral cavity	20	
Oropharyngeal	9	
Hypopharyngeal	3	
Larynx	7	
Treatment duration	19,2 (6,6)	5 - 32

Table 1: Demographic and clinical characteristics of the 39 patients included in the genomic profiling study.

Materials and Methods

RNA extraction

Tumor tissue biopsies were collected both before and after treatment and were snap frozen in liquid nitrogen. The biopsy content was checked on slides stained with hemalun eosin. If the biopsy contained only tumor cells, the biopsy was directly dissolved in lysis buffer and RNA was extracted with RNeasy Mini Kit (Qiagen®). If the biopsy contained also normal cells, then 10 slides of 10 µm thick were prepared and stained and tumoral cells were scraped and dissolved in lysis buffer as previously described.

The quantity and quality of RNA were checked with a bioanalyser Agilent 2100 Expert.

Target preparation and microarray hybridization

1 µg total RNA, prepared from each of the biopsy samples (table xx), was used to generate biotinylated cRNA following the Affymetrix standard protocol using their single cycle amplification kit (Part Number 900493; Affymetrix, Inc.; Santa Clara, CA,

http://www.affymetrix.com/support/technical/manual/expression_manual.affx). 15 µg cRNA was hybridized for 16 h at 45°C to Human Genome U133A GeneChip® oligonucleotide arrays, which carry probes representing >22,000 well-characterized transcripts, from the Human Unigene database (Build 133). Following hybridization, arrays were washed and stained with streptavidin-phycoerythrin and thereafter scanned using an Affymetrix GeneChip Scanner 3000 according to the manufacturer's protocols (Affymetrix); signal intensities were calculated automatically by GCOS.

Data Analysis

Signal intensities were normalized using a quantile-quantile method. All normalized data were log₂-transformed prior to analysis to down-weight the influence of high expression values.

Probe-sets called "absent" or "marginal" by the Affymetrix MAS5 algorithm in all 39 samples were removed from further analysis. In total 5589 probe-sets out of 22283 (25,1%) were removed.

Two different statistical models were applied to identify genes predictive of response. Both models were fitted independently to each of the 16694 probe-set.

The first model is a linear model that takes normalized gene expression data as outcome variable and Response status as predictor. The corresponding p-value evaluates the hypothesis that there is no difference in mean gene expression data between the responder and non-responder groups.

The second approach is a logistic model with response as the outcome variable and normalized gene expression as predictor. The corresponding p-value evaluates the hypothesis that there is no linear relationship between the logarithm of the odd of being a responder and normalized gene expression value..

The following statistical criteria were applied to identify the predictive makers : both p-values are below 0.05 and the absolute difference in mean expression between responder and non responder is greater than 0.263, on logarithmic scale of base 2. These criteria generated a list of 407 probesets, 188 upregulated in non responders and 219 upregulated in responders. Among these, 20 probesets were selected for the current patent. The choice of these 20 probesets was based on:

- The absolute difference in mean expression between responder and non responder
- The biological relevance of the gene based on its function within the cell.

Table 2: Markers based on comparing “Responders” to “Non Responders”.

Responders were defined as patients with tumor shrinkage of more than 25% were arbitrarily considered as responders.

Column 1 is the Affymetrix identifier for the probe-set. Column 2 is the GenBank accession number of the corresponding gene sequence. Column 3 is the corresponding official gene name. Column 4 is the corresponding adjusted mean fold change in expression level between “responder” and “non responder”. Column 5 is the p-value for the test of difference in expression level between “responders” and “non responders”. Column 6 is the 95% confidence interval for the adjusted mean fold change in expression level.

10

Affymetrix Probe Set ID	GenBank Seq. Id. No.	Gene Symbol	Gene	Adjusted Mean Fold Change	P-value logistic response	CI 95 %
202967_at	NM_001512 Seq. Id. No. 1	GSTA4	glutathione s-transferase A4	-2.09830	0.00392	-3.5, -1.26
209283_at	NM_001885 Seq. Id. No. 2	CRYAB	crystallin	-1.94225	0.02137	-3.53, -1.07
39729_at	NM_005809 Seq. Id. No. 3 NM_181738 Seq. Id. No. 4	PRDX2	peroxiredoxin 2	-1.92772	0.00082	-2.73, -1.36
209230_s_at	NM_012385 Seq. Id. No. 5 NM_001042483 Seq. Id. No. 6	NUPR1	p8 protein (candidate of metastasis 1)	-1.92183	0.00380	-2.94, -1.25
210827_s_at	NM_004433 Seq. Id. No. 7	ELF3	e74-like factor 3 (ets domain transcription factor)	-1.89160	0.02704	-3.33, -1.07
202017_at	NM_000120 Seq. Id. No. 8	EPHX1	epoxide hydrolase 1	-1.85643	0.00917	-3.04, -1.13
213400_s_at	NM_005647 Seq. Id. No. 9	TBL1X	transducin (beta)-like 1x-linked	-1.82094	0.00537	-2.78, -1.19
209380_s_at	NM_001023587 Seq. Id. No. 10 NM_005688 Seq. Id. No. 11	ABCC5	ATP-binding cassette, subfamily C (CFTR/MRP), member 5	-1.74913	0.02688	-2.96, -1.04
203973_s_at	NM_005195 Seq. Id. No. 12	CEBPD	ccaat/enhancer binding protein (c/ebp)	-1.71531	0.00684	-2.56, -1.15
208794_s_at	NM_003072 Seq. Id. No. 13	SMARCA4	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4	-1.70440	0.00023	-2.25, -1.29

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Affymetrix Probe Set ID	GenBank Seq. Id. No.	Gene Symbol	Gene	Adjusted Mean Fold Change	P-value logistic response	CI 95 %
202804_at	NM_004996 Seq. Id. No. 14 NM_019862 Seq. Id. No. 15 NM_019898 Seq. Id. No. 16 NM_019899 Seq. Id. No. 17 NM_019900 Seq. Id. No. 18 NM_019901 Seq. Id. No. 19 NM_019902 Seq. Id. No. 20	ABCC1	ATP-binding cassette, sub-family C (CFTR/MRP), member 1	-1.65798	0.02226	-2.65, -1.04
205258_at	NM_004753 Seq. Id. No. 21	INHBB	inhibin	-1.57486	0.01376	-2.41, -1.03
203120_at	NM_001031685 Seq. Id. No. 22 NM_005426 Seq. Id. No. 23	TP53BP2	tumor protein p53 binding protein	-1.47100	0.00016	-1.8, -1.2
216396_s_at	NM_004879 Seq. Id. No. 24 NM_001007277 Seq. Id. No. 25	EI24	etoposide induced 2.4 mrna	-1.44473	0.00133	-1.81, -1.15
201110_s_at	NM_003246 Seq. Id. No. 26	THBS1	thrombospondin 1	2.07501	0.01476	1.2, -3.59
212190_at	NM_006216 Seq. Id. No. 27	SERPINE2	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 2	2.25459	0.02410	1.1, -4.64
222108_at	NM_181847 Seq. Id. No. 28	AMIGO2	adhesion molecule with ig-like domain 2	2.35916	0.02738	1.12, - 4.97
218717_s_at	NM_018192 Seq. Id. No. 29	LEPREL1	leprecan-like 1	2.36758	0.02982	1.13, -4.95
209946_at	NM_005429 Seq. Id. No. 30	VEGFC	vascular endothelial growth factor c	2.38294	0.01717	1.23, -4.63
211538_s_at	NM_021979 Seq. Id. No. 31	HSPA2	heat shock 70kda protein 2	2.44000	0.00310	1.36, -4.38

NB: the “Adjusted Mean Fold Change” is negative when the intensity of the probe set is higher in non responders compared to responders and positive when the intensity is higher in responders compared to non responders.

Claims

1. An *in vitro* method of predicting the response of a cancer patient to treatment with an EGFR inhibitor comprising: determining the expression level of at least one gene selected from the group consisting of GSTA4, CRYAB, PRDX2, NUPR1, ELF3, EPHX1, TBL1X, ABCC5, CEBPD, SMARCA4, ABCC1, INHBB, TP53BP2, EI24 in a tumour sample of a patient and comparing the expression level of the at least one gene to a value representative of an expression level of the at least one gene in a non responding patient population, wherein a lower expression level of the at least one gene in the tumour sample of the patient is indicative for a patient who will respond to the treatment.

2. An *in vitro* method of predicting the response of a cancer patient to treatment with an EGFR inhibitor comprising: determining the expression level of at least one gene selected from the group consisting of THBS1, SERPINE2, AMIGO2, LEPREL1, VEGFC, HSPA2 in a tumour sample of a patient and comparing the expression level of the at least one gene to a value representative of an expression level of the at least one gene in a non responding patient population, wherein a higher expression level of the at least one gene in the tumour sample of the patient is indicative for a patient who will respond to the treatment.

3. The method of claim 1 or 2, wherein the expression level is determined by microarray technology.

4. The method of claims 1 to 3, wherein the expression level of at least two genes is determined.

5. The method of claims 1 to 4, wherein the expression level of at least three genes is determined.

6. The method of claims 1 to 5, wherein the EGFR inhibitor is erlotinib.

7. The method of claims 1 to 6, wherein the cancer is head and neck squamous-cell carcinoma (HNSCC).

8. Use of a gene selected from the group consisting of GSTA4, CRYAB, PRDX2, NUPR1, ELF3, EPHX1, TBL1X, ABCC5, CEBPD, SMARCA4, ABCC1, INHBB, TP53BP2, EI24, THBS1, SERPINE2, AMIGO2, LEPREL1, VEGFC, HSPA2 for predicting the response of a cancer patient to EGFR inhibitor treatment.

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9. The use of claim 8, wherein the cancer is head and neck cancer.

10. The use of claim 8 or 9, wherein the EGFR inhibitor is erlotinib.

10 11. A method of treating a cancer patient identified by a method of claims 1 to 7 comprising administering an EGFR inhibitor to the patient.

12. The method of claim 11, wherein the EGFR inhibitor is erlotinib.

15 13. The method of claim 11 or 12, wherein the cancer is head and neck squamous-cell carcinoma (HNSCC).

Fig. 1

